EcoPURE Total RNA Kit

50 rxns

Cat No: E2075

Shipping : Ship at ambient temperature.
Storage : Store the kit between 15°C and 25°C.

General Information

EcoPURE Total RNA Kit is designed as a simple and convenient purification of high quality total RNA including small RNAs (e.g. microRNAs) from whole blood, cultured cells, and frozen or fresh tissues. This kit utilizes chaotropic ions and silica-based membrane technology, eliminating the need for expensive resins, hazardous phenol-chloroform extractions, β-mercaptoethanol, or time-consuming alcohol precipitation. The standard protocol lasts less than 10 minutes at room temperature and purified RNA can be effectively used in routine downstream applications including cDNA synthesis, northern blotting, differential display, primer extension, and mRNA selection.

Kit Contents

EcoPURE Lysis/Binding Buffer	(22 ml)
EcoPURE Wash Buffer 1	(22 ml)
EcoPURE Wash Buffer 2*	(8 ml concentrate)
EcoPURE Elution Buffer	(5 ml)
EcoPURE Columns	(50)
EcoPURE Collection Tubes	(50)

*Add 32 ml absolute ethanol

Protocol for Whole Blood Total RNA

Each isolation procedure is suitable for isolation of total RNA from 100 µl of non-coagulating fresh whole blood collected using EDTA as the anti-coagulant. If extraction of total RNA from higher volumes of whole blood is required, use *EcoSpin* Blood Total RNA Kit (Cat No: E2090) of EcoTech Biotechnology to isolate total RNA from up to 8 ml of whole blood.

1. Transfer up to 100 µL of non-coagulating fresh blood to an RNase-free microcentrifuge tube (not provided).

2. Add 400 µl *EcoPURE* Lysis/Binding Buffer to each 100 µl whole blood sample. Mix well by pipetting. Then, vortex for 10 seconds until the color of the mixture turns to brownish black.

3. Add 400 µl absolute (96-100%) ethanol to the lysate and mix well by vortexing for 10 seconds.

4. Insert an *EcoPURE* Column into a Collection Tube and transfer 700 µl sample from step 3 to the *EcoPURE* Columns. Centrifuge at maximum speed in a tabletop microcentrifuge 30 seconds at room temperature. Depending on your lysate volume, repeat Step 4 as necessary.

Optional: EcoTech's Total RNA Kit isolates total RNA with minimal amounts of genomic DNA contamination. However, an optional On-Column DNA Removal might be applied for maximum removal of residual DNA that may affect sensitive downstream applications.

5. Discard the flow through and add 400 µl *EcoPURE* Wash Buffer 1 to the *EcoPURE* Column. Centrifuge at maximum speed in a tabletop microcentrifuge 30 seconds at room temperature.

6. Discard the flow through and add 500 µl *EcoPURE* Wash Buffer 2 to the *EcoPURE* Column. Centrifuge at maximum speed in a tabletop microcentrifuge 30 seconds at room temperature.

7. Discard the flow through and add 200 µl *EcoPURE* Wash Buffer 2 to *EcoPURE* Column at maximum speed for 2 minutes to completely remove any residual wash buffer.

8. Transfer the *EcoPURE* Column to a clean RNase-free 1.5 mL microcentrifuge tube (not provided).

9. Add 30-50 µL of *EcoPURE* Elution Buffer to the center of the *EcoPURE* Column membrane and incubate the column at room temperature for 1 minute.

10. Centrifuge at maximum speed in a tabletop microcentrifuge 30 seconds at room temperature.

11. Discard the *EcoPURE* Column and store the purified RNA at -20°C (for a few days) or -80°C (for long term storage) until use.

For further information;
ecotechbiotech.com
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Protocol for Suspension or Adherent Total RNA

Each isolation procedure is suitable for isolation of total RNA from 10^4 to $2x10^6$ cells. If extraction of total RNA from more cells is required, scale up the amounts of reagents used in the entire protocol proportionally.

1a. Suspension Cell Cultures: Pellet appropriate number of suspension cells for 5 minutes at 1200 rpm.

1b. Adherent Cell Cultures: Trypsinize and pellet appropriate number of adherent cells for 5 minutes at 1200 rpm before lysis.

Note: Cell pellets can be stored at -80°C for later use. Frozen pellets should be stored maximally for 2 weeks to ensure that the integrity of the RNA is not compromised. Frozen cell pellets should not be thawed prior to beginning of the protocol. Frozen cell pellets should be immediately lysed with *EcoPURE* Lysis/Binding Buffer.

2. Add 400 µl *EcoPURE* Lysis/Binding Buffer to pelleted cells. Mix well by pipetting. Then, vortex for 10 seconds until the entire pellet is completely dissolved before proceeding to the next step.
Precaution: *EcoPURE* Lysis/Binding Buffer has the ability to inactivate the RNases in cells, however, 4 µl β-mercaptoethanol might be added to each 400 µl *EcoPURE* Lysis/Binding Buffer for cells known to have high RNase activity to ensure the inactivation of RNases.

3. Add 400 µl absolute (96-100%) ethanol to the lysate and mix well by vortexing for 10 seconds.

Optional: For $>1x10^6$ cells, the lysate is recommended to be passed through a 25 gauge needle attached to a syringe 5-10 times at this point, in order to shear the genomic DNA prior to loading onto the column.

4. Insert an *EcoPURE* Column into a Collection Tube and transfer 700 µl sample from step 3 to the *EcoPURE* Columns. Centrifuge at maximum speed in a tabletop microcentrifuge 30 seconds at room temperature. Depending on your lysate volume, repeat Step 4 as necessary.

Optional: EcoTech's Total RNA Kit isolates total RNA with minimal amounts of genomic DNA contamination. However, an optional On-Column DNA Removal might be applied for maximum removal of residual DNA that may affect sensitive downstream applications.

5. Discard the flow through and add 400 µl *EcoPURE* Wash Buffer 1 to the *EcoPURE* Column. Centrifuge at maximum speed in a tabletop microcentrifuge 30 seconds at room temperature.

6. Discard the flow through and add 500 µl *EcoPURE* Wash Buffer 2 to the *EcoPURE* Column. Centrifuge at maximum speed in a tabletop microcentrifuge 30 seconds at room temperature.

7. Discard the flow through and add 200 µl *EcoPURE* Wash Buffer 2 to *EcoPURE* Column at maximum speed for 2 minutes to completely remove any residual wash buffer.

8. Transfer the *EcoPURE* Column to a clean RNase-free 1.5 mL microcentrifuge tube (not provided).
9. Add 50-100 µL of *EcoPURE* Elution Buffer to the center of the *EcoPURE* Column membrane and incubate the column at room temperature for 1 minute.

10. Centrifuge at maximum speed in a tabletop microcentrifuge 30 seconds at room temperature.
11. Discard the *EcoPURE* Column and store the purified RNA at -20°C (for a few days) or -80°C (for long term storage) until use.

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Protocol for Tissue Total RNA

Each isolation procedure is suitable for isolation of total RNA from up to 30 mg fresh or frozen tissue. If extraction of total RNA from more tissue is required, scale up the amounts of reagents used in the entire protocol proportionally.

1a. Cut the tissue sample and determine the amount of tissue by weighing. Grind the sample in a mortar that contains an appropriate amount of liquid nitrogen to cover the sample. Grind the tissue thoroughly using a pestle. Allow the liquid nitrogen to evaporate, without allowing the tissue to thaw. Immediately, add 400 µl *EcoPURE* Lysis/Binding Buffer to grinded tissue. Transfer the grinded and homogenized tissue sample into a RNase-free 1.5 ml microcentrifuge tube (not provided). Mix well by vortexing for 10-15 seconds.

1b. Cut the tissue sample and determine the amount of tissue by weighing. Transfer the tissue sample into a RNase-free 1.5 ml microcentrifuge tube (not provided). Add 400 µl *EcoPURE* Lysis/Binding Buffer. Immediately and vigorously homogenize using a conventional rotor-stator homogenizer with a stainless steel probe at 15,000 rpm for 30 seconds.

Precaution: *EcoPURE* Lysis/Binding Buffer has the ability to inactivate the RNases in cells, however, 4 µl β-mercaptoethanol might be added to each 400 µl *EcoPURE* Lysis/Binding Buffer for cells known to have high RNase activity to ensure the inactivation of RNases.

Optional: The lysate is recommended to be passed through a 25 gauge needle attached to a syringe 5-10 times at this point, in order to shear the genomic DNA and decrease the viscosity prior to loading onto the column.

2. Spin the lysate for 2 minutes at maximum speed in a tabletop microcentrifuge at room temperature to pellet any cell debris. Transfer the supernatant to another 1.5 ml RNase-free microcentrifuge tube. Note the volume of the supernatant.

3. Add equal amount of absolute (96-100%) ethanol to the supernatant and mix well by vortexing for 10 seconds.

4. Insert an *EcoPURE* Column into a Collection Tube and transfer 700 µl sample from step 3 to the *EcoPURE* Columns. Centrifuge at maximum speed in a tabletop microcentrifuge 30 sec at room temperature. Depending on your lysate volume, repeat Step 4 as necessary.

Optional: EcoTech's Total RNA Kit isolates total RNA with minimal amounts of genomic DNA contamination. However, an optional On-Column DNA Removal might be applied for maximum removal of residual DNA that may affect sensitive downstream applications.

5. Discard the flow through and add 400 µl *EcoPURE* Wash Buffer 1 to the *EcoPURE* Column. Centrifuge at maximum speed in a tabletop microcentrifuge 30 seconds at room temperature.

6. Discard the flow through and add 500 µl *EcoPURE* Wash Buffer 2 to the *EcoPURE* Column. Centrifuge at maximum speed in a tabletop microcentrifuge 30 seconds at room temperature.

7. Discard the flow through and add 200 µl *EcoPURE* Wash Buffer 2 to *EcoPURE* Column at maximum speed for 2 minutes to completely remove any residual wash buffer.

8. Transfer the *EcoPURE* Column to a clean RNase-free 1.5 mL microcentrifuge tube (not provided).

9. Add 50-100 µL of *EcoPURE* Elution Buffer to the center of the *EcoPURE* Column membrane and incubate the column at room temperature for 1 minute.

10. Centrifuge at maximum speed in a tabletop microcentrifuge 30 seconds at room temperature.

11. Discard the *EcoPURE* Column and store the purified RNA at -20°C (for a few days) or -80°C (for long term storage) until use.

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